interruptedly during the growth of corn, while the fat seems to be formed last.

3. The calorific determination is of little service in determining the value of corn for feeding purposes.

4. The acid-hydrolyzed proteins do not reveal any unusual features. The diamines are lower than results reported by others, whereas the monoamines of nearly matured corn are higher, but this is not significant in the present research.

5. The total nitrogen of soft corn is only slightly lower than that of mature corn, but the amide nitrogen is much higher.

6. The amide nitrogen, together with the degree of acidity, may serve as a basis for grading corn. Moldy corn contains a large amount of nitrogen in the amide form.

7. Glutelin is the most abundant protein in corn, zein is next, and globulins, albumens, etc., are present in smallest amount.

8. Since zein appears to be present in smaller amount in soft than in mature corn, and since glutelin, globulins, albumens, etc., are present to about the same per cent. in both mature and soft corn, it would seem that zein is formed last, and amide is formed at the expense of zein.

LAFAYETTE, INDIANA.

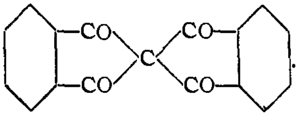
[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF THE COTTON COLLEGE, GAUHATI.]

DIKETOHYDRINDENE. III.[1]

BY ANANDA KISORE DAS AND BROJENDRA NATH GHOSH.

Received April 23, 1919.

It has been shown by one of us with Sastry[2] that the methylene hydrogen atoms in 1,3-diketohydrindene are very reactive, since they condense readily with aromatic aldehydes giving benzylidene derivatives. This led us to think that diketohydrindene could readily condense with anhydrides, such as phthalic anhydride, to give compounds of the type



(I)

Preliminary experiments were therefore conducted, and it was found that the compound obtained was different from the ones noted in the literature. Thus it was noted that Carmelo Marchese[3] had condensed phthalic anhydride and diketohydrindene in the presence of acetic anhydride, and

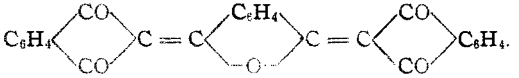
---

[1] For Parts I and II see *Trans. Chem. Soc.*, **107**, 1442 (1915) and **109**, 175 (1916).

[2] *Trans. Chem. Soc.*, **107**, 1442 (1915).

[3] *Gazz. chim. ital.*, **37**, 303–309 (1907).

that the product gave an intensely red solution with alkaline hydroxides. He gives as its formula

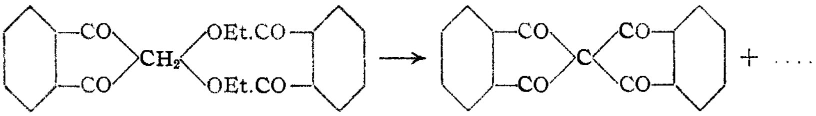


$$C_6H_4\left\langle\begin{matrix}CO\\CO\end{matrix}\right\rangle C=C\left\langle\begin{matrix}C_6H_4\\O\end{matrix}\right\rangle C=C\left\langle\begin{matrix}CO\\CO\end{matrix}\right\rangle C_6H_4. \qquad (II)$$

(II)

The compound we obtained by condensing diketohydrindene and phthalic anhydride in the presence of conc. sulfuric acid, though it melts at nearly the same temperature as the compound of Marchese, is different in other respects; for example, it is not at all soluble in alkaline hydroxide; from nitrobenzene solution it separates immediately in golden yellow plates, whereas his substance is very soluble in nitrobenzene and comes out, in well-defined needles, only on the addition of alcohol; on boiling with aniline our substance separates unchanged and contains no nitrogen; his substance did not separate from aniline, but on pouring the solution into dil. sulfuric acid a black crystalline compound precipitated, differing from the original substance, but not containing any nitrogen. The exact chemical nature of his substance we do not know; it appears to be some degradation product of the original compound. It is, however, a pure substance, as it gives a definite melting point. But we do not know whether decomposition was effected by the aniline or by the sulfuric acid, and so we cannot state definitely the way in which the molecule has been changed.

From the percentage composition we find at any rate that the substance obtained by condensing the phthalic anhydride and diketohydrindene is not the same as given in Formula I. Having failed to prepare the desired compound in this way, we next tried the action of sodium alcoholate on a mixture of diketohydrindene and diethyl-phthalate on the assumption that the reaction would proceed in the following manner:



But after conducting the reaction as described below, we obtained a substance which resembled in every way benzoyl-β,γ-diketohydrindene obtained by E. Schwerin[1] from ethyl-phthalate and acetophenone. It is probable that the desired compound was obtained, but was not stable under the conditions of the experiment, because of the number of carbonyl groups attached to one carbon atom, and so hydrolyzed to *o*-carboxy-benzoyldiketohydrindene, which again decomposed, giving off carbon dioxide with the production of benzoyl-diketohydrindene. In this reaction a little anhydro-*bis*-diketohydrindene[2] is also formed which

---

[1] *Ber.*, **27**, 104–114 (1894).

[2] Wislicenus and Kotzle, *Ann.*, **252**, 76, 77 (1889).

can easily be separated from the benzoyl-diketohydrindene by the slight solubility of the former compound in alcohol. That it is the *bis* compound has been proved by its properties as well as by analysis.

We also tried the condensation of phthalic anhydride, benzoic anhydride and succinic anhydride with diketohydrindene, these 3 being taken to represent 3 classes of anhydrides. Phthalic anhydride is a typical closed chain aromatic anhydride; benzoic anhydride has an open chain, and succinic anhydride is a closed chain aliphatic anhydride. The conditions of the experiment were kept practically uniform. The 3 substances obtained resemble each other very much in their percentage compositions, but their properties, such as solubilities in various solvents, are different. They are, without exception, difficultly soluble in all ordinary solvents; they do not form anilides; they are very high melting and complex substances. It is true that two molecules of diketohydrindene first condensed to form anhydro-*bis*-diketohydrindene[1] and then the anhydro substance condenses with the respective anhydrides giving complex substances. It has been proved that the same substance is produced by condensing phthalic anhydride with either diketohydrindene or anhydro-*bis*-diketohydrindene.

Experimental.

2-Benzoyl-1,3-diketohydrindene.—0.7 g. of sodium was dissolved in 20 cc. of absolute alcohol, the solution was thoroughly cooled, and to it was slowly added 2 g. of 1,3-diketohydrindene dissolved in a little alcohol. The mixture turned red, 3.1 g. of diethyl-phthalate was then added to it and the whole boiled under a reflux condenser on a water bath for 5 to 6 hours. The intensely red solution, after standing overnight, deposited a small quantity of solid, which dissolved on the addition of water. A current of carbon dioxide was passed through the solution, which was then extracted with ether. The aqueous solution on acidification gave a crystalline solid which crystallized from alcohol in almost colorless needles. This was shown by analysis, melting point, and other properties to be benzoyl-diketohydrindene obtained by Schwerin.[1]

Calc.: C, 76.8; H, 4.0. Found: C, 76.9; H, 4.

To confirm this we also prepared the semicarbazone by boiling an alcoholic solution with an excess of an aqueous alcoholic solution of semicarbazide hydrochloride containing sodium acetate. A colorless substance precipitated,

Subs., 0.0985: N_2 (29° and 760 mm.), 10 cc. Calc. for $C_{17}H_{13}O_3N_3H_2O$: N, 12.3. Found: 12.2.

In this reaction a small quantity of a yellow crystalline substance, almost insoluble in alcohol, was obtained. This was crystallized from ace-

---

[1] *Loc. cit.*

tone, and, as indicated by its melting point, and by analysis, as well as by other properties, was evidently anhydro-*bis*-diketohydrindene, obtained by Wislicenus and Kotzle.

Calc.: C, 78.8; H, 3.6. Found: C, 78.4; H, 4.0.

Condensation of Diketohydrindene and Phthalic Anhydride.—2 g. of diketohydrindene and 2.2 g. of phthalic anhydride were mixed and 30 cc. of conc. sulfuric acid added. The mixture was warmed to 50–60° on a water bath, forming a dark brown solution. The heating was continued for an hour, and the mixture after being cooled was poured into cold water when a yellow mass separated. This was dried on porous earthenware, and then recrystallized from nitrobenzene.

Subs., 0.1983: CO_2, 0.6077; H_2O, 0.0669.
Calc. for $C_{25}H_{12}O_3$: C, 83.3; H, 3.3. Found: C, 83.5; H, 3.7.

This substance crystallizes in fine, lustrous flakes, melting at 320° (uncorr.). It is soluble in hot nitrobenzene and aniline; sparingly soluble in xylene, and insoluble in alcohol, acetone, benzene, petroleum ether, and in sodium hydroxide solution. It does not form an anilide. It dissolves in conc. sulfuric acid, giving a brown solution which is not fluorescent. The solution in aniline has a green color. The substance does not sublime on heating.

Anhydrophthalyl-*bis*-1,3-diketohydrindene, II.—This substance was prepared by the method given by Marchese.[1] It was mixed with an excess of aniline and the mixture boiled under a reflux condenser for about an hour. The solution thus obtained was cooled and poured into dil. hydrochloric acid. The black substance which separated was recrystallized from dil. alcohol, giving black prisms which, when heated in a capillary tube, contracted at about 85° and melted to a clear liquid at 110°. The substance is not an anilide because it does not contain any nitrogen.

Subs., 0.1017: CO_2, 0.3029; H_2O, 0.0139.
Calc. for $(C_8H_6O)_x$: C, 81.2; H, 5.08. Found: C, 81.2; H, 5.3.

Condensation of Diketohydrindene and Benzoic Anhydride.—A mixture of 1.46 g. of diketohydrindene, 2.26 g. of benzoic anhydride, and 25 cc. of conc. sulfuric acid, was heated on a water bath at 50° for an hour and then poured into cold water. The substance which separated was collected, washed, dried, and recrystallized from nitrobenzene.

Subs., 0.1504: CO_2, 0.4603; H_2O, 0.0478.
Calc. for $C_{25}H_{12}O_3$: C, 83.3; H, 3.3. Found: C, 83.4; H, 3.5.

The substance crystallizes in golden yellow needles which sublime on heating and melt to a clear liquid only at a very high temperature (above 320°). It is more soluble in nitrobenzene than the previous compound and separates only slowly from its solution.

---

[1] *Loc. cit.*

Condensation of Diketohydrindene and Succinic Anhydride.—A similar product was obtained by condensing 1.5 g. of diketohydrindene and one g. of succinic anhydride in presence of 30 cc. of conc. sulfuric acid, and warming the mixture for one hour at 50°. On pouring into water a greenish mass was obtained, which was recrystallized from nitrobenzene. The substance separates very slowly, requiring sometimes the addition of alcohol. It does not melt even at 316° but sublimes on heating, and is insoluble in alcohol, acetic acid, acetone and benzene. Its solution in aniline is greenish in color, and the substance obtained by pouring the solution into dil. hydrochloric acid did not contain any nitrogen.

Subs., 0.1245: CO_2, 0.3776; H_2O, 0.0391.

Calc. for $C_{21}H_{10}O_2.H_2O$: C, 83.1; H, 3.3. Found: C, 82.7; H, 3.5.

Our thanks are due to Principal Südmersen for the kind interest he has taken in the work.

ASSAM, INDIA.

[CONTRIBUTION FROM THE STANFORD MEDICAL SCHOOL.]

A METHOD OF TISSUE ANALYSIS: APPLIED TO THE POSTERIOR AND ANTERIOR LOBES OF CATTLE PITUITARIES.

BY C. G. MACARTHUR.

Received April 24, 1919.

The cattle pituitaries used in these experiments, were obtained from a local packer. They had been collected over a period of several days and kept frozen until ready for analysis. After freeing the pituitary from bone, connective tissue, fat, etc., it was separated into anterior and posterior lobes. Exactly 10 g. of skin-free, ground material from each lobe was weighed in conical flasks and treated as described under "Method."

Method of Analysis.

The method of analysis used is based on the separation into proteins, lipins and extractives that has already been proven satisfactory.[1] The original method has been simplified, extended to new determinations, and been made available for much smaller amounts of material. The amounts of tissue usually used (10 g.) are probably the most satisfactory, but most of the determinations described can be carried out on 2 g., though with somewhat less accuracy. It will be noticed that the new methods for small quantities are applied in some cases. In others it will be seen that the usual quantitative methods are made available and accurate by decreasing the volume of solutions used to about $^1/_{10}$, avoid-

---

[1] W. Koch, "Methods for the Quantitative Chemical Analysis of Animal Tissue," THIS JOURNAL, 31, 1329 (1909); M. L. Koch and C. Voegtlin, "Chemical Changes in the Central Nervous System in Pellagra," *Hygienic Lab. Bull.*, 103, 51 (1916).